TOWNSEND
and
TOWNSEND
and
CREW
LLP

Denver, Colorado
Tel 303 571-4000

Palo Alto, California
Tel 650 326-2400

Seattle, Washington
Tel 206 467-9600

San Francisco

Two Embarcadero Center
Eighth Floor
San Francisco
California 94111-3834
Tel 415 576-0200
Fax 415 576-0300

6 January 2004

VIA EXPRESS MAIL, WITH RETURN POSTCARD ENCLOSED

PCT International Application Processing Div.
USPTO International Division
Assistant Commissioner for Patents
Mail Stop PCT
PO Box 1450
Alexandria, VA 22313-1450

Re: International Application No. PCT/US03/18373
Title: ANTI-CD30 STALK AND ANTI-CD30 ANTIBODIES SUITABLE FOR USE IN IMMUNOTOXINS
Applicant: THE GOVERNMENT OF THE UNITED STATES, AS REPRESENTED BY THE SECRETARY OF THE DEPARTMENT OF HEALTH AND HUMAN SERVICES *et al.*
International Filing Date: 9 June 2003
Express Mail Label No.: EV 330 850 327 US
Date of Mailing: 6 January 2004
Our File No.: 15280-4641PC

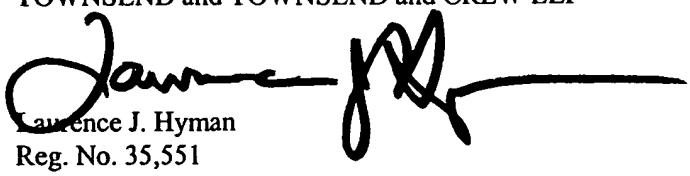
Dear Officer:

Enclosed are the Chapter II Demand and thirteen (13) substitute specification pages 4-6, 9, 11, 12, 14, 82-84, 88, 90 and 90a submitted as an Article 34 Amendment for the above-referenced patent application. The only changes were insertions of SEQ ID:NOs. and corrections of typographical errors that do not include matter which go beyond the disclosure in the international application as filed.

Thank you for your attention to this matter.

Respectfully submitted,

TOWNSEND and TOWNSEND and CREW LLP



Laurence J. Hyman
Reg. No. 35,551

LJH/nan
Enclosures: Chapter II Demand
Thirteen (13) substitute specification pages 4-6, 9, 11, 12, 14, 82-84, 88, 90 and 90a
Twenty-one (21) pages of Sequence Listing
Diskette and Statement
Transmittal Letter and Postcard

60111197 v1

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ US

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: 15280-4641PC

International application No.	International filing date (*day/month/year*)	(Earliest) Priority date (*day/month/year*)
PCT/US03/18373	9 June 2003 (09.06.03)	7 June 2002 (07.06.02)

Title of invention

ANTI-CD30 STALK AND ANTI-CD30 ANTIBODIES SUITABLE FOR USE IN IMMUNOTOXINS

Box No. II APPLICANT(S)

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

THE GOVERNMENT OF THE UNITED STATES, AS REPRESENTED BY THE SECRETARY OF THE DEPARTMENT OF HEALTH AND HUMAN SERVICES
Office of Technology Transfer
6011 Executive Boulevard, Suite 325
Rockville, Maryland 20852-3084
United States of America

Telephone No.: 301.496.7056
Facsimile No.: 301.402.0220
Teleprinter No.:
Applicant's registration No. with the Office:

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
US	US

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

PASTAN, Ira, H.
11710 Beall Mountain Road
Potomac, Maryland 20854
United States of America

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
US	US

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

NAGATA, Satoshi
6070 California Circle 413
Rockville, Maryland 20852
United States of America

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
JP	US

☒ Further applicants are indicated on a continuation sheet.

International application No.
PCT/US03/18373

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet should not be included in the demand.

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

ONDA, Masanori
320 Lorraine Drive
Rockville, Maryland 20852
United States of America

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
JP	US

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

NUMATA, Yoshito
2-451-1-311 Hirano Kashiwara
Osaka 581-0019
Japan

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
JP	JP

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

SANTORA, Kenneth
10543 Wheatley Street
Kensington, Maryland 20895
United States of America

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
US	US

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

BEERS, Richard
5306 Russett Road
Rockville, Maryland 20853
United States of America

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
US	US

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

KREITMAN, Robert
11915 Reynolds Avenue
Potomac, Maryland 20854
United States of America

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
US	US

Name and address: (*Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*)

SINHA, Abhishek
11533 Aliento Court
San Diego, California 92127
United States of America

State (*that is, country*) of nationality:	State (*that is, country*) of residence:
US	US

International application No.
PCT/US03/18373

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is ☒ agent ☐ common representative

and ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.

☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

HYMAN, Laurence, J.
TOWNSEND AND TOWNSEND AND CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
United States of America

Telephone No.: 415.576.0200

Facsimile No.: 415.576.0300

Teleprinter No.:

Agent's registration No. with the Office: 35,551

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination **to start on the basis of**:

☒ the international application as originally filed

the description	☐	as originally filed
	☒	as amended under Article 34
the claims	☐	as originally filed
	☐	as amended under Article 19 (together with any accompanying statement)
	☒	as amended under Article 34
the drawings	☒	as originally filed
	☐	as amended under Article 34

2. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. ☐ The applicant wishes the start of the international preliminary examination **to be postponed** until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: ENGLISH

☒ which is the language in which the international application was filed.

☐ which is the language of a translation furnished for the purposes of international search.

☐ which is the language of publication of the international application.

☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby **elects all eligible States** *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes **not to elect**:

International application No.
PCT/US03/18373

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

				For International Preliminary Examining Authority use only	
				received	not received
1.	translation of international application	:	sheets	☐	☐
2.	amendments under Article 34	:	13 sheets	☐	☐
3.	copy (or, where required, translation) of amendments under Article 19	:	sheets	☐	☐
4.	copy (or, where required, translation) of statement under Article 19	:	sheets	☐	☐
5.	letter	:	1 sheet	☐	☐
6.	other (*specify*)	:	sheets	☐	☐

The demand is also accompanied by the item (s) marked below:

1. ☒ fee calculation sheet
2. ☐ original separate signed power of attorney
3. ☐ original general power of attorney;
4. ☐ copy of general power of attorney; reference number, if any:
5. ☐ statement explaining lack of signature
6. ☒ sequence listing in computer readable form
7. ☐ tables in computer readable form related to sequence listings
8. ☒ other (*specify*) Transmittal Letter, Article 34 Amendment with thirteen (13) substitute specification pages 4-6, 9, 11, 12, 14, 82-84, 88, 90 and 90a; Twenty-one (21) pages of Sequence Listing, Statement and Diskette; Postcard

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

X _____

Lawrence J. Hyman
TOWNSEND AND TOWNSEND AND CREW LLP
USPTO Reg. No.: 35,551
Applicants' Agent

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

CHAPTER II

PCT

FEE CALCULATION SHEET

Annex to the Demand

For International Preliminary Examining Authority use only

International application No. PCT/US03/18373	
Applicant's or agent's file reference 15280-4641PC	Date stamp of the IPEA

Applicant

THE GOVERNMENT OF THE UNITED STATES, AS REPRESENTED BY THE SECRETARY OF THE DEPARTMENT OF HEALTH AND HUMAN SERVICES *et al.*

CALCULATION OF PRESCRIBED FEES

1. Preliminary examination fee	490.00	P
2. Handling fee (*Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.*)	172.00	H
3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box	662.00 TOTAL	

MODE OF PAYMENT

- ☒ authorization to charge deposit account with the IPEA (see below)
- ☐ cash
- ☐ cheque
- ☐ revenue stamps
- ☐ postal money order
- ☐ coupons
- ☐ bank draft
- ☐ other (*specify*):

AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT
(*This mode of payment may not be available at all IPEAs*)

The IPEA/ US

☒ is hereby authorized to charge the total fees indicated above to my deposit account.

☒ (*this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit*) is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

20-1430	6 January 2004	Laurence J. Hyman
Deposit Account Number	Date (*day/month/year*)	Signature

See Notes to the fee calculation sheet

compensate for loss of some of the immunotoxin by binding by free sCD30 in the extracellular fluids, such as the serum. This problem also extends to other immunoconjugates, such as a radioisotope attached to an antibody, to the extent that their cell killing or labeling abilities are reduced by binding to free sCD30 in the circulation. There remains a need in the art for immunotoxins directed against the CD30 antigen which have high cytotoxicity to target cells or which bind to intact CD30 but not to sCD30.

BRIEF SUMMARY OF THE INVENTION

[11] It has now been discovered that a residual, extracellular "stalk" of CD30 remains after cleavage of sCD30. The stalk provides an advantageous and previously unrecognized target for immunotoxins. The invention provides antibodies that bind to the CD30 stalk or to epitopes destroyed upon the cleavage of CD30 which results in the stalk. The invention further relates to the discovery of new anti-CD30 antibodies that form effective immunotoxins and are particularly suitable for making disulfide stabilized Fv ("dsFv")-immunoconjugates.

[12] In particular, this invention provides antibodies that bind specifically to a stalk of CD30 of a cell, or to an epitope destroyed upon cleavage of sCD30 from intact CD30. The antibody fragment can be an Fab, a recombinant single chain variable region, or a disulfide stabilized recombinant variable region ("dsFv"). In particularly preferred embodiments, the antibody or fragment thereof is a dsFv. In some embodiments, the antibody binds to a peptide consisting of residues 329 to 379 of CD30. In other embodiments, the antibody binds to a peptide consisting of residues 339 to 379 of CD30. In yet other embodiments, the antibody binds to a peptide consisting of residues 349 to 379 of CD30. In still other embodiments, the antibody binds to a peptide consisting of residues 359 to 379 of CD30. In still other embodiments, the antibody binds to a peptide consisting of residues 369 to 379 of CD30. In still other embodiments, the antibody binds to an epitope of CD30 mapping to residues 329 to 379 of CD30. In still other embodiments, the antibody binds to an epitope of CD30 mapping to residues 339 to 379 of CD30. In still other embodiments, the antibody binds an epitope of CD30 mapping to residues 349 to 379 of CD30. In still other embodiments, the antibody binds to an epitope of CD30 mapping to residues 359 to 379 of CD30. In still other embodiments, the antibody binds to an epitope of CD30 mapping to residues 369 to 379 of CD30. If the antibody binds an epitope that spans the cleavage site or

is discontinuous but binds to both sCD30 and to the stalk, it is preferable that the antibody does not increase the rate of cleavage of sCD30 from the intact CD30. Preferably, the antibody binds to an epitope mapping to Epitopes IIa or VI of CD30. In preferred forms, the antibody has one or more complementarity determining regions as shown in Figures 2a and 2b for antibody T105 or of T201.

[13] In another group of embodiments, the invention provides composition comprising any of the antibodies described above, attached to a therapeutic moiety. Typically, the antibody is attached to the therapeutic moiety by conjugation or by fusion (that is, the antibody-therapeutic moiety is expressed as a recombinant protein). In some embodiments, the therapeutic moiety is selected from the group consisting of a cytotoxin, a drug, a radioisotope, or a liposome loaded with a drug or a cytotoxin. In some embodiments, where the therapeutic moiety is a cytotoxin, the cytotoxin is selected from the group consisting of ricin A, abrin, ribotoxin, ribonuclease, saporin, calicheamycin, diphtheria toxin, a *Pseudomonas* exotoxin ("PE"), and botulinum toxins A through F. In some embodiments in which the cytotoxin is a PE, the PE can be selected from the group consisting of PE35, PE38, PE38KDEL, PE40, PE4E, and PE38QQR. The compositions described above can further comprise a pharmaceutically acceptable carrier.

[14] In another group of embodiments, the invention provides for the use of an anti-CD30 antibody that binds specifically to a stalk of CD30 of a cell, or to an epitope destroyed upon cleavage of sCD30 from intact CD30, for the manufacture of a medicament to inhibit the growth of a CD30+ cancer cell. In some embodiments, the antibody can be selected from the group consisting of an scFv, dsFv, a Fab, or a $F(ab')_2$. In preferred embodiments, the antibody is a dsFv. The invention further provides for the use of a composition for the manufacture of a medicament for inhibiting growth of a CD30+ cancer cell, which composition comprises an antibody as just described conjugated or fused to a therapeutic moiety. In some embodiments, the therapeutic moiety is selected from the group consisting of a cytotoxin, a drug, a radioisotope, or a liposome loaded with a drug or a cytotoxin. In some embodiments where the therapeutic moiety is a cytotoxin, the cytotoxin can be ricin A, abrin, ribotoxin, ribonuclease, saporin, calicheamycin, diphtheria toxin, a *Pseudomonas* exotoxin ("PE"), or a botulinum toxin selected from A through F. In some embodiments, the cytotoxin is a PE. In some embodiments where the cytotoxin is a PE, the PE is PE35, PE38, PE38KDEL, PE40, PE4E, or PE38QQR. In some preferred embodiments, the PE is PE38.

[15] In yet another group of embodiments, the invention provides nucleic acids encoding an antibody that binds specifically to a stalk of CD30 of a cell, or to an epitope destroyed upon cleavage of sCD30 from intact CD30. The antibody can be, for example, an scFv, a dsFv, a Fab, or a $F(ab')_2$. In particularly preferred embodiments, the antibody is a dsFv. The nucleic acid can further encode a polypeptide which is a therapeutic moiety. The therapeutic moiety can be a drug or a cytotoxin. In some embodiments, the cytotoxin can be ricin A, abrin, ribotoxin, ribonuclease, saporin, calicheamycin, diphtheria toxin, a *Pseudomonas* exotoxin ("PE"), or a botulinum toxin selected from A through F. In some preferred embodiments, the cytotoxin is a PE. In some embodiments where the cytotoxin is a PE, the PE is PE35, PE38, PE38KDEL, PE40, PE4E, or PE38QQR. In some preferred embodiments, the PE is PE38.

[16] The invention further provides expression vectors comprising any of the nucleic acids described above operably linked to a promoter.

[17] In another set of embodiments, the invention provides methods of inhibiting growth of a CD30+ cancer cell by contacting said cell with an antibody that binds specifically to a stalk of CD30 of a cell, or to an epitope destroyed upon cleavage of sCD30 from intact CD30, which antibody is fused or conjugated to a therapeutic moiety, which therapeutic moiety inhibits growth of said cell. The antibody can be, for example, an scFv, a dsFv, a Fab, or a $F(ab')_2$. In particularly preferred embodiments, the antibody is a dsFv. The therapeutic moiety can be a cytotoxin, a drug, a radioisotope, or a liposome loaded with a drug or a cytotoxin. In some embodiments where the therapeutic moiety is a cytotoxin, the cytotoxin can be ricin A, abrin, ribotoxin, ribonuclease, saporin, calicheamycin, diphtheria toxin, a *Pseudomonas* exotoxin ("PE"), or a botulinum toxin selected from A through F. In some preferred embodiments, the cytotoxin is a PE. In some embodiments where the cytotoxin is a PE, the PE is PE35, PE38, PE38KDEL, PE40, PE4E, or PE38QQR. In some preferred embodiments, the PE is PE38.

[18] In yet another group of embodiments, the invention provides anti-CD30 antibodies, wherein said antibodies comprise a sequence of at least one complementarity determining region ("CDR") shown in Figures 2a and b, of a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:14, SEQ ID NO:15, SEQ ID

cells of the biological sample with an anti-CD30 antibody selected from the group consisting of: an antibody that binds specifically to a stalk of CD30 of a cell, or to an epitope destroyed upon cleavage of sCD30 from intact CD30, and an antibody having at least one complementarity determining region as shown in Figure 2 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:22, SEQ ID NO:29, SEQ ID NO:38, and SEQ ID NO:39, the antibody being fused or conjugated to a detectable label; and, (b) detecting the presence or absence of said label, wherein detecting the presence of the label indicates the presence of a CD30+ cell in said sample. In some embodiments, the antibody is selected from the group consisting of an scFv, a dsFv, a Fab, or a $F(ab')_2$.

[24] In yet another group of embodiments, the invention provides antibodies useful for inducing complement-dependent cytotoxicity. In this regard, the invention provides antibodies having at least one complementarity determining region (CDR) from a variable heavy chain or variable light chain selected from the group consisting of SEQ ID NO:6, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:21, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:40, and SEQ ID NO:41. In preferred embodiments, the antibodies have the CDRs of a variable heavy chain and a variable light chain selected from the group consisting of: (a) SEQ ID NO:6, and SEQ ID NO:21 (antibody T24), (b) SEQ ID NO:11 and SEQ ID NO:26 (antibody T420), (c) SEQ ID NO:12 and SEQ ID NO:27 (antibody T427), (d) SEQ ID NO:13 and SEQ ID NO:28 (antibody T405), and (e) SEQ ID NO:40 and SEQ ID NO:41 (antibody T408). In preferred forms, the variable heavy and variable light chains have the sequences of these antibodies, preferably with mutations in the framework region which "humanize" them. The invention further provides compositions comprising any of the antibodies just described and a pharmaceutically acceptable carrier.

[25] The invention further provides for the use of any of the antibodies described in the preceding paragraph for the manufacture of a medicament to inhibit the growth of cancer cells expressing CD30. Additionally, the invention provides methods for inhibiting the growth of cancer cells expressing CD30, said method comprising administering to a patient having a CD30-expressing cancer a therapeutically effective amount of an antibody having at least one CDR of a variable heavy chain or variable light chain selected from the group consisting of SEQ ID NO:6, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:21, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:40, and SEQ ID NO:41, as shown in Figures 2a and b. Preferably, the method comprises administering to a

SEQ ID NO:40 and SEQ ID NO:41 (antibody T408). The invention also provides host cells expressing an isolated nucleic acid encoding an antibody having variable heavy and variable light chains selected from the group consisting of (a) SEQ ID NO:6, and SEQ ID NO:21 (antibody T24), (b) SEQ ID NO:11 and SEQ ID NO:26 (antibody T420), (c) SEQ ID NO:12 and SEQ ID NO:27 (antibody T427), (d) SEQ ID NO:13 and SEQ ID NO:28 (antibody T405), and (e) SEQ ID NO:40 and SEQ ID NO:41 (antibody T408).

[28] The invention further provides kits for detecting the presence of a CD30+ cancer cell in a biological sample. The kits comprise(a) a container, and (b) an anti-CD30 antibody selected from the group consisting of: an antibody that binds specifically to a stalk of CD30 of a cell, or to an epitope destroyed upon cleavage of sCD30 from intact CD30, and an antibody that has at least one complementarity determining region having a sequence shown in Figure 2 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:22, SEQ ID NO:29, SEQ ID NO:38, and SEQ ID NO:39, which anti-CD30 antibody is fused or conjugated to a detectable label. In some embodiments, the antibody in the kit is selected from the group consisting of an scFv, a dsFv, a Fab, or a $F(ab')_2$.

BRIEF DESCRIPTION OF THE DRAWINGS

[29] **Figure 1**. Figure 1 is a cartoon showing six epitopes of CD30 as determined by competition binding experiments; the epitopes are designated by Roman numerals. The Figure shows a cell membrane (thick dark line at bottom of figure), with a representation of the CD30 molecule, showing the transmembrane and extracellular domains. The putative site at which sCD30 is cleaved from the stalk is shown by an arrow. Epitopes IIa and VI were determined to be non-shed epitopes. Ber-H2, HRS-4, Ki-4, HeFi-I, Ki-1, and M67 are previously known anti-CD30 antibodies. Antibodies designated with a "T" were generated in the course of the present invention. The relative size of the font for each antibody roughly corresponds with the affinity of the antibody for CD30. The location of each epitope was mapped by the following observations: 1) Epitopes ("Ep") II, III, IV are close to each other because some members of them showed cross competition for binding to CD30, 2) Ep I and III are a little correlated to each other for the same reason, 3) Ep I, III and IV are far from the

cleavage site, 4) Ep IIb and V are close to the cleavage site, 5) Ep IIa and VI are non-shed epitopes or located on the cleavage site. 6)Ep I and Ep IIb are on or near the CD30L binding site. The position of the peptide chain is a representation. Underlined antibodies were tested as immunotoxins in an early series of studies. The plasma membrane of the cell is represented as a black curved line at bottom of figure. The extracellular portion of CD30 is positioned in the Figure above the line representing the plasma membrane, with the intracellular portion below the line representing the plasma membrane. As defined herein, the stalk is the portion of CD30 molecule from the first amino acid on the cell-proximal side of the cleavage site to the last amino acid on the extracellular side of the plasma membrane.

[30] **Figures 2a and 2b**. Figures 2a and 2b set forth the amino acid sequences of the variable regions of various anti-CD30 MAbs. Figure 2a sets forth the sequences of the variable heavy chain ("VH") (SEQ ID NOS:2-14, 38 and 40), while Figure 2b sets forth the sequences of the respective variable light ("VL") chains (SEQ ID NOS:15-29, 39 and 41). The MAbs are identified in the left hand column. The next column sets forth the SEQ ID NO: for the amino acid sequence of the VH or VL, respectively, of the antibody named in the left hand column. DNA sequences of each VH or VL of the MAbs were determined by a RACE method and the deduced amino acid sequences were aligned according to their Kabat numbering. The calculated position of antigen complementarity determining regions (CDRs) and framework regions (FRs), based on alignments using the Kabat numbering system, are indicated in the first line of each panel. A subclone encoding a truncated VL sequence derived from SP2/O myeloma cells (Carroll et al., *Mol. Immunol.*, **25**:991-995 (1988)) was obtained from T13 and T14 hybridoma, the sequence of which was omitted for simplification. CL2 (Rozemuller et al., *Int. J. Cancer*, **92**:861-870 (2001)) and Ki-4 (Klimka et al., *Br. J. Cancer*, **80**:1214-1222 (1999)) are previously known anti-CD30 Fv sequences.

[31] **Figures 3a-f.** Figure 3 encompasses six figures, denoted as Figures 3a to 3f, respectively. (Figures 3a to 3c are positioned in the top row, from left to right, Figures 3d to 3f are positioned in the bottom row and are likewise positioned in order from left to right). Figures 3a to 3f show FACS analysis of selected anti-CD30(dsFv)-PE38 immunotoxins on CD30-positive cells. The immunotoxins at various concentrations were incubated with L540 cells. The cell-bound immunotoxins were detected by anti-PE rabbit antibody and phycoerythrin-labeled anti-rabbit IgG. The five solid lines in each histogram from the left to right represent the staining with 0, 0.01, 0.1, 1, and 10 μg/ml of immunotoxins, respectively. For easier comparison, the areas of the staining with 1 μg/ml of immunotoxin are filled with

[34] **Figures 6a and 6b**. Figures 6a and 6b show complement-dependent cytotoxicity of intact antibodies assayed on two CD30+ cell lines, Karpas299 and L540, a Hodgkin's Disease cell line, as described in Example 6. The legend shows the symbols assigned to the antibodies tested. HeFi-1 and AC10 are antibodies known in the art. LeY designates an antibody that recognizes the LewisY antigen, used as a negative control. "Cell+C' " designates cell plus complement only, meaning that antibody was not added to that culture, as a further control, while "C' " indicates complement only.

[35] **Figures 7a and 7b**. These graphs present the results of studies of the cytotoxicity of various immunotoxins made from antibodies of the invention. Figure 7a shows the cytotoxicity of antibodies T6, T104, T201, and T408 on a cell line (A431) transfected to express CD30. Figure 7b shows the cytotoxicity of the same immunotoxins on the same cell line, transfected to express the irrelevant antigen CD25 as a negative control.

[36] **Figures 8a, 8b and 8c**. These graphs present the results of studies of the cytotoxicity of various immunotoxins made from antibodies of the invention. Figure 8a shows the cytotoxicity of antibodies T6, T13, T105, and T201 on an anaplastic large cell lymphoma cell line, SUDHL1. Figure 8b shows the cytotoxicity of the same immunotoxins on a Hodgkin's Disease cell line called L540. Figure 8C shows the cytotoxicity of these immunotoxins on a cell line, Ramos, that does not express CD30, as a negative control.

DETAILED DESCRIPTION

I. INTRODUCTION

A. Discovery Of CD30 Stalk And Antibodies That Bind To It

[37] CD30 was discovered in 1982, and was cloned in 1992. Durkop et al., Cell, 68:421-427 (1992), identified CD30 as a 595 amino acid protein with an 18 residue leader sequence, a 365 amino acid extracellular domain, a single transmembrane domain of 24 residues, and an intracellular domain of 188 residues. The amino acid sequence of CD30 was identified in the Durkop et al. publication. Durkop et al. identify the transmembrane domain as commencing on the N-terminal side with a proline at residue 380. The C-terminus of the extracellular domain is identified as a lysine at position 379, which may thus be considered as the amino acid of CD30 most proximal to the cell surface.

WHAT IS CLAIMED IS:

1. An isolated antibody that binds specifically to a stalk of CD30 of a cell, or to an epitope destroyed upon cleavage of soluble CD30 ("sCD30") from intact CD30.

2. An antibody of claim 1, wherein said antibody is selected from the group consisting of an Fab, a single chain variable region ("scFV"), and a disulfide stabilized recombinant variable region ("dsFv").

3. An antibody of claim 1, which binds to a peptide selected from the group consisting of: residues 329 to 379 of CD30, residues 339 to 379 of CD30, residues 349 to 379 of CD30, residues 359 to 379 of CD30, and residues 369 to 379 of CD30.

4. An antibody of claim 1, which binds to an epitope of CD30 mapping to Epitope IIa or Epitope VI of CD30.

5. An antibody of claim 4, which has the complementarity determining regions ("CDRs") of antibody T105, as shown in Figures 2a and b.

6. An antibody of claim 1, which has the complementarity determining regions ("CDRs") of antibody T201, as shown in Figures 2a and b.

7. A composition comprising an antibody of claim 1, conjugated or fused to a therapeutic moiety.

8. A composition comprising an antibody of claim 3, conjugated or fused to a therapeutic moiety.

9. A composition comprising an antibody of claim 4, conjugated or fused to a therapeutic moiety.

10. A composition comprising an antibody of claim 5, conjugated or fused to a therapeutic moiety.

11. A composition comprising an antibody of claim 6, conjugated or fused to a therapeutic moiety.

12. A composition of claim 7, wherein the therapeutic moiety is selected from the group consisting of a cytotoxin, a drug, a radioisotope, or a liposome loaded with a drug or a cytotoxin.

13. A composition of claim 8, wherein the therapeutic moiety is selected from the group consisting of a cytotoxin, a drug, a radioisotope, or a liposome loaded with a drug or a cytotoxin

14. A composition of claim 9, wherein the therapeutic moiety is selected from the group consisting of a cytotoxin, a drug, a radioisotope, or a liposome loaded with a drug or a cytotoxin.

15. A composition of claim 10, wherein the therapeutic moiety is selected from the group consisting of a cytotoxin, a drug, a radioisotope, or a liposome loaded with a drug or a cytotoxin.

16. A composition of claim 11, wherein the therapeutic moiety is selected from the group consisting of a cytotoxin, a drug, a radioisotope, or a liposome loaded with a drug or a cytotoxin.

17. A composition of claim 15, wherein the cytotoxin is selected from the group consisting of ricin A, abrin, ribotoxin, ribonuclease, saporin, calicheamycin, diphtheria toxin, a *Pseudomonas* exotoxin, and botulinum toxins A through F.

18. A composition of claim 12, wherein the cytotoxin is selected from the group consisting of ricin A, abrin, ribotoxin, ribonuclease, saporin, calicheamycin, diphtheria toxin, a *Pseudomonas* exotoxin, and botulinum toxins A through F.

19. A composition of claim 18, wherein said *Pseudomonas* exotoxin is selected from the group consisting of PE35, PE38, PE38KDEL, PE40, PE4E, and PE38QQR.

20. A composition of claim 7, further comprising a pharmaceutically acceptable carrier.

21. A use of an anti-CD30 antibody that binds specifically to a stalk of CD30 of a cell, or to an epitope destroyed upon cleavage of sCD30 from intact CD30, for the manufacture of a medicament to inhibit the growth of a CD30+ cancer cell.

22. A use of claim 21, wherein said antibody is selected from the group consisting of an scFv, dsFv, a Fab, or a $F(ab')_2$.

23. A use of a composition, which composition comprises an antibody of claim 1 conjugated or fused to a therapeutic moiety, for the manufacture of a medicament for inhibiting growth of a CD30+ cancer cell.

24. A use of claim 23, wherein the therapeutic moiety is selected from the group consisting of a cytotoxin, a drug, a radioisotope, or a liposome loaded with a drug or a cytotoxin.

25. A use of claim 24, wherein said cytotoxin is a *Pseudomonas* exotoxin.

26. A use of claim 25, wherein the *Pseudomonas* exotoxin is PE38.

27. A nucleic acid encoding an antibody that binds specifically to a stalk of CD30 of a cell, or to an epitope destroyed upon cleavage of sCD30 from intact CD30.

28. A nucleic acid of claim 27, wherein said antibody binds to an epitope of CD30 selected from Epitope IIa and VI.

29. A nucleic acid of claim 27, further wherein said nucleic acid encodes a polypeptide which is a therapeutic moiety.

30. An expression vector comprising a nucleic acid of claim 27 operably linked to a promoter.

31 An expression vector comprising a nucleic acid of claim 28, operably linked to a promoter.

32. An expression vector comprising a nucleic acid of claim 29 operably linked to a promoter.

33. A method of inhibiting growth of a CD30+ cancer cell by contacting said cell with an antibody that binds specifically to a stalk of CD30 of a cell,

60. A method of claim 59, wherein said antibody is a dsFv.

61. A method of claim 59, wherein said therapeutic moiety is selected from the group consisting of a cytotoxin, a drug, a radioisotope, or a liposome loaded with a drug and a cytotoxin.

63. A method of claim 61, wherein the cytotoxin is selected from the group consisting of ricin A, abrin, ribotoxin, ribonuclease, saporin, calicheamycin, diphtheria toxin, a *Pseudomonas* exotoxin, and botulinum toxins A through F.

64. A method for detecting the presence of a CD30+ cell in a biological sample, said method comprising:

(a) contacting cells of said biological sample with an anti-CD30 antibody selected from the group consisting of: an antibody that binds specifically to a stalk of CD30 of a cell, or to an epitope destroyed upon cleavage of sCD30 from intact CD30, and an antibody having at least one complementarity determining region as shown in Figure 2 of a variable heavy chain or variable light chain selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:22, SEQ ID NO:29, SEQ ID NO:38 and SEQ ID NO:39, said antibody being fused or conjugated to a detectable label; and,

(b) detecting the presence or absence of said label,

wherein detecting the presence of said label indicates the presence of a CD30+ cell in said sample.

65. A method of claim 64, wherein said antibody is selected from the group consisting of an scFv, a dsFv, a Fab, or a $F(ab')_2$.

66. An antibody having at least one variable heavy chain or variable light chain selected from the group consisting of SEQ ID NO:6, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:21, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:40, and SEQ ID NO:41.

67. An antibody of claim 66, wherein said antibody has a variable heavy chain and a variable light chain selected from the group consisting of: (a) SEQ ID NO:6, and SEQ ID NO:21 (antibody T24), (b) SEQ ID NO:11 and SEQ ID NO:26 (antibody T420), (c)

therapeutically effective amount of antibody having at least one complementarity-determining region ("CDR") of a mouse monoclonal antibody designated as AC10.

76. A method of claim 75, wherein the CDRs of the variable heavy and variable light chains of said antibody are as in antibody AC10.

77. A method of claim 76, wherein the variable heavy and variable light chains of said antibody are as in antibody AC10.

78. An isolated nucleic acid encoding an antibody having the complementarity determining regions ("CDRs") of variable heavy and variable light chains selected from the group consisting of (a) SEQ ID NO:6, and SEQ ID NO:21 (antibody T24), (b) SEQ ID NO:11 and SEQ ID NO:26 (antibody T420), (c) SEQ ID NO:12 and SEQ ID NO:27 (antibody T427), (d) SEQ ID NO:13 and SEQ ID NO:28 (antibody T405), and (e) SEQ ID NO:40 and SEQ ID NO:41 (antibody T408).

79. An isolated nucleic acid encoding an antibody having variable heavy and variable light chains selected from the group consisting of (a) SEQ ID NO:6, and SEQ ID NO:21 (antibody T24), (b) SEQ ID NO:11 and SEQ ID NO:26 (antibody T420), (c) SEQ ID NO:12 and SEQ ID NO:27 (antibody T427), (d) SEQ ID NO:13 and SEQ ID NO:28 (antibody T405), and (e) SEQ ID NO:40 and SEQ ID NO:41 (antibody T408).

80. A host cell expressing an isolated nucleic acid encoding an antibody having variable heavy and variable light chains selected from the group consisting of (a) SEQ ID NO:6, and SEQ ID NO:21 (antibody T24), (b) SEQ ID NO:11 and SEQ ID NO:26 (antibody T420), (c) SEQ ID NO:12 and SEQ ID NO:27 (antibody T427), (d) SEQ ID NO:13 and SEQ ID NO:28 (antibody T405), and (e) SEQ ID NO:40 and SEQ ID NO:41 (antibody T408).

81. A kit for detecting the presence of a CD30+ cancer cell in a biological sample, said kit comprising:

(a) a container, and

(b) an anti-CD30 antibody selected from the group consisting of: an antibody that binds specifically to a stalk of CD30 of a cell, or to an epitope destroyed upon

cleavage of sCD30 from intact CD30, and an antibody that has at least one complementarity determining region having a sequence shown in Figures 2 and 6 of SEQ ID